and is recommended when cases of *Aspergillus* infection are detected but they are not recommended on routine basis. Surveillance cultures of high risk patients, in search of *Aspergillus* or *Candida*, may guide the administration of preemptive or prophylactic antifungals, However, the real efficacy of this surveillance practices, remains to be defined.

S11 – Is malareia being rolled back

TuS3 Malaria vaccine development: Approaches and status

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Malaria remains one of the world most deadly diseases. The World Health Organization estimates that there are 300 to 500 million clinical cases of malaria annually, causing 1.1 to 1.7 million deaths. The vast majority of deaths occurs among children under 5 years of age and 90% of malaria cases are in sub-Saharan Africa.

While various control measures can contribute to ameliorate this situation, it is generally accepted that, in the long run, vaccines are likely to be the most effective and cost efficient way to fight this disease. Fortunately, there exist today convincing preclinical and preliminary clinical data suggesting that the development of a malaria vaccine is needed feasible.

In the first part of this presentation, we will describe the various approaches researchers in this field have employed in their attempts to develop vaccines targeting the different stages of the *Plasmodium falciparum* parasite life cycle. The rationale and results obtained with each approach will be described.

We will then focus on one promising malaria vaccine candidate, designated RTS, S/SBAS2, that has been developed by SmithKline Beecham Biologicals and its collaborators. This vaccine has recently yielded unprecedented efficacy results in clinical trials conducted both under laboratory and natural (field) challenge conditions. Avenues for further development and improvement of this vaccine will be described.

TuS4 Transmission blocking drugs or vaccines for control of malaria

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The rate of malaria transmission is an important denominator in the epidemiology of clinical of malaria. Malaria parasites are distributed in the population by Plasmodium-infected Anopheles mosquitoes; the number of infected mosquitoes can vary significantly per geographical area. The more intense the transmission, the younger the age at which malaria immunity and protection from lethal disease is acquired. Infectiousness of the human host to mosquitoes is determined by the presence of sexual stages (gametocytes [gct]) in the circulation. Transmission-blocking (TB)-vaccines or drugs aim at a reduction of the human infectious reservoir by killing or inactivating gct's and form an important component in malaria control. TB vaccines and drugs can directly reduce morbidity and mortality but also may act as an adjunct to other malaria vaccines by reducing the spread of resistant strains. TB-immunity induced by vaccines is antibody-mediated and a number of target molecules on the parasite membrane have been identified. Some recombinant proteins are in the process of production for clinical testing.

TB-drugs include artemisinin, primaquine and its derivative tafenoquine. These drugs are primarily used for treatment or prophylaxis, but also show potent killing effects on gct's. The effect of transmission reductions or clinical malaria will depend on the rate of transmission and is strongly geographically determined.

S12 – Pharmacodynamics of antifungals

TuS5 Development of resistance during antifungal therapy

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In the last decade emergence of resistance during antifungal treatment has become a well recognized problem in HIV-infected patients receiving long term prophylaxis and treatment with fluconazole for oropharyngeal candidiasis. Molecular typing revealed that in most cases development of resistance of the original *C. albicans* strain is encountered, but coexistence of different subpopulations exhibiting various degrees of resistance have also been demonstrated. Risk factors identified for the development of fluconazole resistance were low CD4+ counts, previous exposure to the drug and a total cumulative dose of at least 10 g. No difference in the occurrence of resistance could be observed in patients receiving continuous versus intermittent therapy. In non-AIDS patients emergence of azole resistant *C. albicans* has been rarely reported. However, an increased incidence of infections due to *Candida* spp. intrinsically resistant to fluconazole such as *C. glabrata* and *C. krusei* has been observed in neutropenic patients as well as in intensive care patients receiving azole drugs for prophylaxis or treatment. In the last years progress has been made in elucidating the molecular mechanisms contributing to resistance development of *Candida* to the azoles which include enhanced expression of multidrug efflux pumps, target enzyme overexpression and point mutation of its encoding gene resulting in decreased affinity to the drug. Recurrences of cryptococcal meningitis in AIDS patients during maintenance therapy with azoles have infrequently been associated with the emergence of azole-resistant strains. Although there are case reports of infections due to amphotericin B resistant yeasts, development of resistance to this antifungal agent appears to be uncommon. Flucytosin resistance is known to develop frequently during monotherapy and is therefore used only in combination.

TuS6 Pharmacodynamics of antifungals

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PK/PD investigations of antibacterials have been used to predict outcomes against both susceptible and resistent pathogens. For example, studies with b-lactama have demonstrated that when dosing achieves drug levels exceeding a T > MIC of at least 40% efficacy is observed against bath susceptible and resistant pathogens. These observations have been important in the development appropriate dosing strategies and susceptibility breakpoints.

Similar study with antifungal drugs has been limited. Recent studies have begun to address several important PK/PD questions. Investigation of the in-vivo time course activity of currently available antifungal classes have found that some demonstrate concentration dependent killing while others do not. In addition, some antifungal compounds have demonstrated prolonged persistent effects or postantifungal effects (PAFE) while others have not. Studies have also demonstrated that different PK/PD parameters predict in-vivo outcomes for these drugs. Fluconazole demonstrated no in-vivo concentration dependent killing against susceptible or resistant C. albicans and in-vivo exposures did, however, result in significant PAFEs. The ratio of the 24 hr AUC/MIC was the PK/PDP that we, a moat closely associated with outcomes. Furthermore, similar efficacy was observed against both susceptible and resistant C. albicans when the magnitude of the 24 hr AUC/MIC exceeded a value of 25. Similarly, study with flucytosine did not demonstrate concentration dependent killing. Flucytosine therapy did not, however, result in prolonged PAFEs. T > MIC was the PK/PDP predictive of efficacy. Amphotericin B demonstrated both concentration dependent killing and prolonged PAFEs. Peak level/MIC was the parameter best predictive of in-vivo outcomes against Candida species.

These initial studies suggest that further PK/PD analysis of current and developmental antifungals can offer important dosing Information for initial clinical trials, effective treatment of resistant pathogens and the development of susceptibility breakpoints.

TuS7 Synergism between antifungals

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Infections caused by fungi are difficult to treat and are increasing. Only a few antimycotic agents are available and partly because of the diversity of fungi, most species are susceptible to only a few of them. Combination therapy is an alternative. The value and/or efficacy of combination therapy can be evaluated in vitro, in vivo models and in human trials. Most studies have been done in vitro by checkerboard type experiments. However, the interpretation is difficult. Recently, models have become available to fit to these type of data, such as the Greco model of interaction and the Bliss model. The results of these models can be used in simulations to determine possible effects in vivo. In order to use these models, data have to be of a quantitative nature. Methods to obtain quantitative readings such as extinction as a measure of antimycotic effect using specific dyes are now available for filamentous fungi. For instance, in vitro synergy has thus been shown for

itraconazole and terbinafine against clinical isolates of *Scedosporium prolificans* Results from animal experiments are still scarce and difficult to interpret because of altered pharmacokinetics in most animals. In humans, synergism seems to be present for amphotericine combined with flucytosine to treat infections caused by *Cryptococcus spp.* It is expected, that new methods to determine synergism between antifungals will result in a more ratinoal approach of using combinations of antimycotics in humans.

S13 – New strategies for treatment of viral infections

TuS9 T cell therapy of HCMV infection

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The reconstitution of HCMV-specific immune responses after allogeneic SCT has been demonstrated to be protective against the development of HCMV disease. S. Riddell and P. Greenberg have shown protective HCMV-specific T cell immunity to be transfered to the recipients of an allogeneic stem cell transplant by the infusion of donor-derived ex vivo generated HCMV-specific cytotoxic T cell (CTL) clones. Alternative strategies to deplete of alloreactive T cells and/or enrich for CMV specific T cells in donor PBMNCs are increasingly explored and will be discussed. One possibility is to pulse dendritic cells, the "professional" antigen presenting cells, with soluble synthetic peptides to induce and propagate HCMV specific CTLs from HCMV-seropositive and also HCMV-seronegative donors. After repetitive specific stimulation T cell lines highly enriched for HCMV specific T cells can be generated and safely transferred to patients. In a phase I/II-study recipients of an allograft with persistent HCMV infection in spite of prolonged antiviral chemotherapy received $1 \times 10^7/m^2$ polyclonal cell lines without any significant side effects. A transient reduction of viral load could be documented in all these patients, the majority of them showed sucessful control of HCMV infection.

TuS11 Ribozyme and stem cell gene therapy for the treatment of HIV infection

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Objectives: Genetic modification of hematopoietic stem cells with anti-HIV catalytic RNAs (Ribozymes).
Methods: Hammerhead rihozymes targeting the HIV-1 *tat* and *rev* transcripts are transduced in the backbone of a murine retroviral vector into primary hetamotpoietic progenitor cells from HIV-1 infected individuals. The ribozymes are expressed as a polycistronic transcript from the LTR of the LN retroviral vector. Our hypothesis is that stable transduction of pluripotent hematopoietic stem cells by ribozyme expressing vectors followed by engraftment of these cells in the marrow, will ultimately provide a population of HIV-1 resistant T-cells, monocytes, and dendritic cells. To test this, we have initiated a clinical trial involving HIV-1 infected individuals who have AIDS related lymphoma using vectors harboring ribozyme or vector backbone alone transduced into their stem cells. In parallel to the clinical studies, we are testing new strategies for ribozyme-target co-localization using chimeric RNA molecules, which harbor our rihozymes of interest tethered to sequences, which direct the ribozymes to discrete intracellular localizations.
Results: The clinical trial studies have demonstrated a selective survival of ribozyme expressing cells within a period of several weeks following bone marrow transplantation and long term engraftment in at least one patient. Our new chimeric ribozyme localization studies demonstrate that a nucleolar-localized ribozyme is a potent inhibitor of HIV-1.
Conclusions: Ribozyme mediated gene therapy for the treatment of HIV-1 infection is feasible utilizing hematopoietic stem cells. Potent inhibition of HIV-1 infection by a nucleolar ribozyme suggests nucleolar trafficking of HIV-1 RNAs, opening new targets for anti-HIV-1 therapies.

S14 – Assesment of respons to antiviral therapy...

TuS13 Assessment of response to antiviral therapy and antiviral resistance: Hepatitis B and C virus

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Chronic viral hepatitis due to hepatitis B virus (HBV) or hepatitis C virus (HCV) infection is a major cause of morbidity and mortality, effecting hundreds of millions of individuals worldwide. Long term complications of untreated chronic viral hepatitis include progressive liver disease and hepatocellular carcinoma. Interferon alpha has historically been the first line treatment for chronic viral hepatitis. However, this therapy is only moderately effective and is often limited by side effects. Newer agents including nucleoside analogs for chronic hepatitis B, and ribavirin in combination with interferon for chronic hepatitis C, have shown promise for improving response rates. In chronic hepatitis B, antigen tests (HBeAg, HBsAg), antibody tests (anti-HBs, anti-HBe) and nucleic acid tests (HBV DNA) are used to monitor response to antiviral therapy, while in chronic hepatitis C, treatment response is monitored by following qualitative and/or quantitative HCV RNA in serum. In general, successful treatment of chronic hepatitis B results in disappearance of the viral antigens and HBV DNA, and seroconversion to positive anti-HBs and anti-HBe status. In chronic hepatitis C, successful treatment is associated with clearance of HCV RNA from serum during the first 3–6 months of therapy, and absence of detectable viral RNA in serum 6 months after cessation of therapy. In both cases, such responses predict sustained clinical remissions and histological improvement of liver disease. Drug resistance to nucleoside analog monotherapy is well documented in chronic hepatitis B, and appears to result from critical mutations within the HBV polymerase gene. The role of viral mutations in determining sensitivity or resistance of HBV and HCV to interferon therapy is less well defined, primarily due to a lack of suitable culture systems for phenotypic characterization of putative resistant viral populations.

TuS16 Detection of drug-resistant HCMV strains by monitoring response to antiviral treatment in immunocompromised patients

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Monitoring of response to treatment by quantitative determination of antigenemia viremia and leukoDNAemia is the most useful tool for early detection of drug-resistant HCMV strains in immunocompromised patients. In fact, lack of reduction or increase in level of measured viral parameters in blood during therapy suggests the unrestricted viral replication. In particular, while antigenemia and leukoDNAemia quantitate viral components, viremia is a direct measure of the amount of infectious virus and of the virus replicative potential during treatment. However, delayed reduction in viral load during treatment is not invariably associated with the emergence of a drug-resistant HCMV strain. Thus, rapid confirmatory assays are needed in order to decide treatment change. A simplified immediate- early plaque-reduction assay using blood leukocytes as inoculum, allows a rapid (4–6 days) screening for drug-resistance. Detection of specific mutations in HCMV UL97 or UL54 genes directly in clinical specimens by PCR-based methods or sequencing allows demonstration of the presence of drug-resistant strains within 2–3 days. In AIDS patients, disseminated HCMV infections have been treated mostly in the presence of specific symptoms, whereas in solid organs or bone marrow recipients adoption of preemptive therapy protocols implies initiation of anti-HCMV treatment when patients are still in the asymptomatic phase of the infection. This difference along with the need in the recent past of prolonged anti-HCMV maintenance treatment in AIDS patients might account for the reported greater prevalence of HCMV drug-resistant strains in this patient population with respect to transplant recipients. However, the recent introduction of potent antiretroviral combination protocols was followed by a drastic reduction in the prevalence of HCMV infections in AIDS patients, with a further reduced number of reported new resistant HCMV strains. In the transplantation settings, several anedoctical reports of HCMV drug-resistant strains points to a possible emerging problem in the near future.